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(57) Abstract

The 3'-end of BC200 RNA, commencing at nucleotide 159 has the sequence: UAAGCGUAAC UUCCCUCAAA GCAACAACCC CCCCCCCCCU UU 42, [SEQ ID NO 2]. Oligonucleotide probes in accordance with the invention are complementary to at least a portion of this sequences such that they bind specifically and selectively to human BC200 RNA. The probes are useful in determining the distribution of BC200 RNA in the body and as an indicator of neoplastic diseases in non-neuronal tissue.

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Description

BC200 RNA, Probes Therefor and Use Thereof

The invention described in this application was made with funding provided by the National Institute of Mental Health under grant number MH38819. The United States may have certain rights to the invention.

Background of the Invention

This application relates to oligonucleotide probes that are specific for human BC200 RNA and to the use of these probes in screening for neoplastic diseases and neurological and psychiatric disorders.

BC200 RNA is a 200-nucleotide long, non-translatable RNA that is prevalently expressed in the nervous system of primates, including man. A partial nucleotide sequence of BC200 RNA from monkey brains was reported by Watson and Sutcliffe, Molecular & Cellular Biology 7, 3324-3327 (1987). This 138 nucleotide sequence showed substantial homology to the Alu left monomer, a sequence that is repeated many times throughout the human and other primate genomes. Watson and Sutcliffe hypothesized that the remainder of the 200-nucleotide RNA may correspond to a poly-(A) tract.

We have now determined the complete sequence for human BC200 RNA and have discovered that the 3'-end of the polynucleotide includes a unique sequence which can be used to specifically detect the presence of human BC200 RNA without interference from other instances of the Alu sequence within the genome. Further, we have discovered that while BC200 RNA does not appear to occur in detectable amounts in normal non-neuronal tissue other than germ cells, it does occur

consistently in high amounts in a variety of non-neuronal human tumor tissues.

Accordingly, it is an object of the present invention to provide oligonucleotide probes that bind specifically with the unique portion of human BC200 RNA or a corresponding chromosomal DNA sequence.

It is a further object of the present invention to provide a method to test for the presence of BC200 RNA in a tissue sample, particularly for purposes of screening for neoplastic diseases and neurological and psychiatric disorders.

It is a further object of the present invention to provide kits useful in testing for the presence of BC200 RNA in a tissue sample.

Summary of the Invention

The 3′-end of BC200 RNA, commencing at nucleotide 159 has the sequence:

UAAGCGUAAC UUCCCUCAAA GCAACAACCC CCCCCCCCCU UU 42

[SEQ ID NO 2]

Oligonucleotide probes in accordance with the invention are complementary to at least a portion of this sequence such that they bind specifically and selectively to human BC200 RNA. The probes are useful in determining the distribution of BC200 RNA in the body and as an indicator of neoplastic diseases in non-neuronal tissue. In addition, probes spanning nucleotides 48 and 49 or the non-A nucleotides of the A-rich region (146-148) of BC200 RNA are useful for detecting the RNA.

Brief Description of the Drawings

Figs. 1A and 1B show the results of Northern Blot experiments to detect BC200 RNA in various human tissues.

Detailed Description of the Invention

Primate BC200 RNA is a 200-nucleotide long non-translatable RNA. We have determined the primary sequence of human BC200 RNA to be as follows:

XXCCGGGCGC GGUGGCUCAC GCCUGUAAUC CCAGCUCUCA GGGAGGCUAA GAGGCGGGAG 60
GAUAGCUUGA GCCCAGGAGU UCGAGACCUG CCUGGGCAAU AUAGCGAGAC CCCGUUCUCC 120
AGAAAAAGGA AAAAAAAAAA CAAAAGACAA AAAAAAAAUA AGCGUAACUU CCCUCAAAGC 180
AACAACCCCC CCCCCCCUUU 200

[SEQ ID NO 1]

The X's at positions 1 and 2 are independently either G or absent.

This primary sequence can be subdivided into three structural domains. Domain I is nucleotides 1-122 and is substantially homologous to Alu repetitive elements which are found in high copy numbers in primate genomes. However, this region includes two bases not found in Alu or SRP-RNA, i.e., nucleotides at positions 48 and 49, which can be used to develop amplification primers specific to BC200 sequences. Domain II is an A-rich region consisting of nucleotides 123-158. Domain III, consisting of nucleotides 159-200, contains a unique sequence, with no homology to other known human sequences, which can be used to identify BC200 RNA in tissues.

In one aspect of the present invention, oligonucleotide probes are provided which are complementary to the unique sequences of Domain III of human BC200 RNA, or to corresponding chromosomal DNA, i.e., which are complementary to at least a portion of the sequence:

UAAGCGUAAC UUCCCUCAAA GCAACAACCC CCCCCCCCCU UU 42

[SEQ ID NO 2]

Such probes are linear oligonucleotides containing from about 10 to 60 bases. The length must be sufficient to provide a reasonable degree of specificity such that

binding with BC200 RNA will be preferred over binding to other polynucleotides.

As used herein, the term "oligonucleotide probe" refers to either a DNA or an RNA probe.

One probe within the scope of the invention is complementary to the nucleotides 156-185 of BC200 RNA. This 30-nucleotide probe has the sequence:

TTGTTGCTTT GAGGGAAGTT ACGCTTATTT 30

[SEQ ID NO 3]

Another useful probe is a 21-nucleotide probe complementary to nucleotides 158-178, i.e.:

TTTGAGGGAA GTTACGCTTA T 21

[SEQ ID NO 7]

As will be apparent from these two examples, suitable probes may be complementary with the portions of BC200 RNA outside Domain III, provided they are also complementary to a portion (i.e., at least about 10 bases) of the unique Domain III sufficient to provide specificity. Probes may also be complementary to portions of Domain III alone. A further aspect of the invention is a second class of probes which are complementary to a portion of Domain II spanning nucleotieds 146-148. These probes may be used for detection of BC200 RNA or as amplification primers.

In a still further aspect of the invention, probes can be utilized which are complementary to and specifically hybridize with a portion of the Alu-repetitive sequence spanning the two unique nucleotides as positions 48 and 49 of BC200 RNA or corresponding DNA. Examples of such probes are:

CCTCTTAGCC TCCCTGAGAG CT 22

[SEQ ID NO 12]

an antisense probe that will bind BC200 RNA and:

CCAGCTCTCA GGGAGGCTAA 20

[SEQ ID NO 13]

a sense probe that will bind to corresponding DNA sequences. These probes can be used for detection or as amplification primers.

The probes of the present invention can be made by any of a variety of techniques known in the art. For example, RNA probes can be generated by in vitro transcription. In this approach, the desired sequence is first cloned into a suitable transcription vector (e.g., pBluescript). This vector is linearized so that transcription will terminate at a specific location, and RNA is transcribed from such linearized templates, using SP6, T3, or T7 RNA polymerase. The probes can be ^{35}S- or ^{3}H-labeled by adding the appropriate radiolabeled precursors to the reaction mixture. Template DNA is then digested with DNase I. RNA probes can be further purified by gel filtration or gel electrophoresis.

Probes can also be made by oligolabeling, although this technique is more suited to longer nucleic acid polymers. In this method, double stranded DNA is first denatured. Random sequence oligonucleotides are then used as primers for the template directed synthesis of DNA. The Klenow fragment of E. coli DNA polymerase I is frequently used in this application. Reverse transcriptase can be used if the template is RNA. Labeling of the probe is achieved by incorporation of radiolabeled nucleotides, e.g., [α-^{32}P]dNTPs.

Another approach for generation of probes is nick translation. Double stranded DNA is used in this method. Nicks (gaps in one strand) are introduced by DNase I. E. coli DNA polymerase I is used simultaneously to add nucleotide residues to the 3′ termini of the DNA at the nick points. Incorporation of radiolabeled precursor nucleotides results in the uniform labeling of the probe. Probes contain both strands.

Single stranded DNA probes can be made from templates derived from bacteriophage M13 or similar vectors. An oligonucleotide primer, complementary to a specific segment of the template, is then used with the Klenow fragment of E. coli DNA polymerase I to generate a radiolabeled strand complementary to the template. The probe is purified for example by gel electrophoresis under denaturing conditions.

Oligonucleotides of any desired sequence can also be synthesized chemically. Solid phase methods are routinely used in the automated synthesis of oligonucleotides.

Probes useful in the invention can be labeled. A variety of enzymes can be used to attach radiolabels (using dNTP precursors) to DNA termini. The 3′ termini of double stranded DNA can for example be labeled by using the Klenow fragment of E. coli DNA polymerase I. Blunt ended DNA or recessed 3′ termini are appropriate substrates. T4 DNA polymerase can also be used to label protruding 3′ ends. T4 polynucleotide kinase can be used to transfer a ^{32}P-phosphate group to the 5′ termini of DNA. This reaction is particularly useful to label single stranded oligonucleotides. Probes can also be labeled via PCR labeling in which labeled nucleic acids and/or labeled primers are used in PCR generation of probes from an appropriate clone. See Kelly et al., Genomics 13: 381-388 (1992).

The probes of the invention can be used to screen tissue for the presence of BC200 RNA. For example, the distribution of BC200 RNA in the human retina was determined, with the result that BC200 RNA was detected in the ganglion cell layer, the inner plexiform layer and the innermost layer of the inner nuclear layer but not in other parts of the retina. Similar mapping was possible in the hippocampus and neocortex used the probes of the invention.

Using the aforementioned probes in in situ hybridization experiments, we have demonstrated that BC200 expression levels are altered in some brain areas of aging individuals and in Alzheimer's brains. In several cortical areas, specifically Brodmann areas 9 and 17/18, BC200 levels were found to decrease with age (age group 50-90 years). In the same brain areas of Alzheimer's patients, a drastic increase of BC200 expression was observed. BC200 probes can therefore be used to differentiate between Alzheimer's disease and normal aging.

Non-neuronal tissues may also be tested for the presence of BC200 RNA in accordance with the invention. We have observed that most normal, non-neuronal tissue does not contain detectable amounts of BC200 RNA, but that a variety of human tumor tissues do express BC200 RNA which can be detected using the probes of the invention. Thus, a further aspect of the invention is a method of screening for neoplastic diseases using probes for BC200 RNA.

The basic methodology of the screening procedure involves the following steps:

(1) obtaining a physiological sample;

(2) treating the sample to render RNA and/or DNA available for hybridization;

(3) hybridizing the treated sample with a probe specific for Domain III of human BC200 RNA; and

(4) analyzing for the occurrence of hybridization. Suitable physiological samples include biopsy specimens, body fluids such as sputum, cervical or esophagal scrapings and skin samples. Neuronal tissue, for example biopsy specimens and post-mortem material, may also be screened for BC200 RNA for assessment of neurological disorders.

While the method employed to treat the tissue sample is not critical, provided that nucleic acids in

the sample are made available for hybridization, several specific options are worth noting. Direct isolation of RNA by the guanidine thiocyanate method followed by CsCl-density gradient centrifugation may be effective in many cases, particularly for isolation of RNA from biopsy specimens. (See Example 4) Where the sample size is small, as in a sputum sample for example, however, amplification of the RNA may be desirable.

Amplification of the RNA can be achieved by first lysing cells in the sample to render RNA available for hybridization. This can be accomplished by (1) extraction of RNA with guanidinium thiocyanate, followed by centrifugation in cesium chloride; (2) extraction of RNA with guanidine HCl and organic solvents; or (3) extraction of RNA with mild detergents (such as NP-40), combined with proteinase digestion. These and other RNA extraction methods are described in Sambrook et al. The isolated RNA is converted into cDNA which is then amplified using probes selective for the 3' end of BC200 sequence. (See Example 3 and U.S. Patent No. 4,683,202 incorporated herein by reference.) cDNA may also be amplified using ligase-based methods (Barany et al., Proc. Nat'l. Acad. Sci. USA 88, 189-193 (1991)) or isothermal transcription-based methods (Kwoh et al., Proc. Nat'l. Acad. Sci. USA 86, 1173-1177 (1989)). The amplified DNA can then be detected directly via an appropriate probe in accordance with the invention.

The hybridization can be carried out using any of the numerous procedures known for assaying for nucleic acids. These include various blot techniques (i.e., dot, Northern, Southern, etc.), and sandwich based techniques such as those described in U.S. Patents Nos. 4,486,539; 4,751,177; 4,868,105; 4,894,325 and European Patent Publication 0 238 332 (all of which are

incorporated herein by reference). To facilitate detection, the probe may have a label, such as a radiolabel, chemiluminescent label, fluorescent label or chromogenic label, or an immobilization moiety. Probes modified with biotin or digoxygenin, which can serve as either a detectable label or an immobilization moiety, are particularly useful.

The probes of the invention are suitably supplied as part of a kit for screening tissue for BC200 RNA. In addition to the probe or other detection reagent that produces a diagnostic reaction product if BC200 RNA is present, such a kit may include one or more of the following:

(1) a solid support to which the diagnostic reaction product nucleic is affixed during the screening procedure;

(2) amplification primers and enzymes for amplification of nucleic acids in a sample;

(3) a labeled reagent that reacts with the diagnostic reaction product to render it detectable; and

(4) solutions effective to lyse the physiological sample to render RNA available for hybridization. Suitable amplification primers include those identified in Example 2, as well as others which will result in amplification, if present, of Domain III of BC200 RNA, possibly together with portions of Domains II and I. A particularly preferred 5'-amplification primer is one that is complementary to a portion of Domain I of BC200 RNA, or the corresponding cDNA, that includes the unique nucleotides at positions 48 and 49. Suitable enzymes include reverse transcriptase, Taq polymerase, rTth DNA polymerase and RNA polymerase.

While the invention is described principally in terms of using oligonucleotide hybridization probes to

detect BC200 RNA, it will be appreciated that the beneficial result of screening for neoplastic diseases can be achieved using any detection technique. For example, RNA-specific antibodies to BC200 RNA could be used, e.g., in an ELISA assay, to detect BC200 RNA in tissue samples. See Uchiumi et al., J. Biol. Chem. 266: 2054-62 (1991). Peptide nucleic acids that hybridize with BC200 RNA may also be used as diagnostic reagents. See Hanvey et al., Science 258: 1481-1485 (1992). Similarly, we have observed that BC200 RNA may be complexed with proteins in vivo to form a ribonucleoprotein ("RNP"). Antibodies specific to BC200-RNP could also be used in an immunoassay detection scheme.

EXAMPLE 1

Determination of Human BC200 RNA Sequence

Total cellular RNA and poly(A)$^+$ RNA were isolated from human neocortex according to the method of Feramisco et al., J. Biol. Chem. 257: 11024-11031 (1982). 5 μg of poly(A)$^+$ RNA were tailed with CTP, using poly A polymerase, and the C-tailed RNA was converted into double-stranded cDNA as described in DeChiara et al., Proc. Nat'l. Acad. Sci. USA 84: 2624-2628 (1987). EcoRI-adaptors (Pharmacia) were attached, according to the instructions of the manufacturer, and cDNA smaller than about 400 base pairs was selected on a 4% polyacrylamide gel. Electro-eluted cDNA was closed into λZAP (Stratagene), following the manufacturer's manual. We screened 3.6×10^4 plaques with an oligonucleotide probe complementary to the sixty 3'-most nucleotides of rat BC1 RNA (DeChiara et al., 1987; Tiedge et al., Proc. Nat'l. Acad. Sci. USA 88: 2093-2097 (1991)) at low stringency (final wash at 35°C in 6 x SSC; 1 x SSC is 150 mM sodium chloride, 15 mM sodium citrate, pH 7.4). Four clones were identified as

positive, and the sequences of their inserts (both strands) were determined using the enzymatic chain termination reaction. Sanger et al., Proc. Nat'l. Acad. Sci. USA 74: 5463-5467 (1977); Toneguzzo et al., Biotechniques 6: 460-469 (1988). Twelve additional clones were later identified and sequenced using the enzymatic chain termination method after rescreening of the library with oligonucleotide probes complementary to specific BC200 RNA sequences, and yielded additional information about Domain I. The resulting sequence based on all of the clones is shown above (SEQ ID NO 1).

EXAMPLE 2

Amplification of BC200 RNA

The 5' and 3' Domains of BC200 RNA were amplified separately.

For amplification of the 5' BC200 sequence, 1 μg total RNA was isolated from human neocortex using the guanidinium thiocyanate method followed by phenol extraction and CsCl centrifugation, and converted into first strand cDNA using the thermostable rTth DNA polymerase (Perkin Elmer Cetus) according to the instructions of the manufacturer. The primer used in this step was

GTTGTTGCTT TGAGGGAAG 19

[SEQ ID NO 4]

The 3' end of the product was then T-tailed using dTTP and terminal transferase (Boehringer Mannheim). The tailed cDNA was PCR-amplified (Frohman et al., Proc. Nat'l. Acad. Sci. USA 85: 8998-9002 (1988)) in 30 cycles (denaturation for 30 s at 94°C, annealing for 1 min at 55°C, extension for 2 min at 72°C; initial denaturation was for 4 min at 94°C, final extension was for 10 min at 72°C), using the primers

GCCTTCGAAT TCAGCACCGA GGGAAGTTAC GCTTA 35

[SEQ ID NO 5]

and

GCCTTCGAAT TCAGCACCAA AAAAAAAAAA AAAAA 35

[SEQ ID NO 6]

The products were further amplified in a second set of 30 cycles (conditions see above), using the adapter primer

GCCTTCGAAT TCAGCACC 18

[SEQ ID NO 8]

After digestion with EcoRI, the PCR-products were cloned into the EcoRI site of λZAPII (Stratagene) following the manual of the manufacturer. 10^3 plaques were screened with an internal oligonucleotide probe

AAAAAAAAA(T/A) (T/G)GCCGGGCGC GGT 23

[SEQ ID NO 9]

and 6 positive clones were sequenced.

For amplification of the 3′ BC200 sequence, 10 μg total RNA from human neocortex were A-tailed using poly A polymerase (DeChiara et al., 1987). Tailed RNA was then converted into first strand cDNA with reverse transcriptase in the presence of MeHgOH (Invitrogen), using the primer

GCCTTCGAAT TCAGCACCTT TTTTTTTTTT TTTTT 35

[SEQ ID NO 10]

This primer, in combination with the primer

GCCTTCGAAT TCAGCACCAA AATAAGCGTA ACTTCCC 37

[SEQ ID NO 11]

was also used for PCR-amplification (see above). Products were cloned into λZAPII (see above), and 14 clones that were identified with SEQ ID NO 11 were sequenced using the enzymatic chain termination reaction.

EXAMPLE 3

Production of BC200 RNA Specific Probe

Two types of probes have routinely been used. In the first example, an oligodeoxynucleotide of the desired sequence was chemically synthesized and purified by chromatography or gel electrophoresis. The oligonucleotide was then radiolabeled by phosphorylation of the 5′ end. This was achieved by using the enzyme polynucleotide kinase with γ^{32}P-labeled ATP. The radiolabeled probe (specific activity: > 10^8 cpm/μg) was separated from unincorporated label by gel filtration, and the probe was used at a concentration of 10^6 cpm/ml.

In the second example, RNA probes were generated by *in vitro* transcription. In this approach, the desired sequence was first cloned into a suitable transcription vector (e.g., pBluescript). This vector was then linearized (so that transcription would terminate at a desired location), and RNA was transcribed from such linearized templates, using SP6, T3, or T7 RNA polymerase. ^{35}S- or ^{3}H-UTP was present during the transcription reaction, and the resulting probes were thus ^{35}S- or ^{3}H-labeled. Template DNA was then digested with DNase I, proteins were phenol-extracted, and the probes were ethanol-precipitated. RNA probes were frequently used for *in situ* hybridization experiments.

EXAMPLE 4

Evaluation of BC200 RNA in Human Tissues

Human biopsy material was obtained that included the tumor tissue itself and adjacent normal tissue from the same organ. Tumor tissue and normal tissue were separated and frozen until further processing. RNA was extracted from such tissue, using the guanidine

thiocyanate method in combination with CsCl-density centrifugation (reference or describe).

For Northern hybridization analysis, 10 μg of total RNA (per sample) were run on a 1.5% agarose gel in the presence of 2.2 M formaldehyde. RNA was transferred to Gene Screen membranes (NEN) and immobilized by UV-illumination. BC200 RNA was detected on such blots by using a probe that recognizes 30 nucleotides in the 3′ unique region of BC200 RNA. (SEQ ID NO 3) Hybridization was at 42°C. Filters were washed in 0.5 x SSC, 0.1% SDS, at 50°C, and exposed for autoradiography.

The results of these experiments are shown in Fig. 1. Fig. 1A reveals expression levels in a variety of tumor and corresponding normal tissues of BC200 RNA (a) and of 7SL RNA (b; 7SL RNA was monitored as a control RNA that is ubiquitously expressed by all cell types). Abbreviations: HB, human brain (normal tissue, loaded as a positive control); HLU, human lung (squamous cell carcinoma); HM, human lung metastatic melanoma; HBt, human breast tumor (adenocarcinoma); HP, human parotid carcinoma; T, tumor tissue; N, adjacent normal tissue from the same organ. Note that in the examples shown in Fig. 1A, BC200 RNA is strongly expressed in non-neural tumor tissue and is at the same time undetectable in the corresponding normal tissue. Fig. 1B includes examples of tumors that do not express BC200 RNA. Abbreviations: HB, human brain (as in A); HLu, human lung (squamous cell carcinoma, as in A); HL, human liver tumor; HBd, human bladder carcinoma; HK, human kidney carcinoma; HC, human colon carcinoma. In situ hybridization experiments have confirmed that in BC200-positive tumors, the RNA is expressed by tumor cells, not however by neighboring non-malignant cells (e.g., stroma cells, inflammatory cells).

A summary of the experimental data on the expression of BC200 RNA in various human tumors is given in Table 1. The symbols +, ++ and +++ reflect increasing levels of RNA detected. Especially noteworthy is the patient-to-patient consistency of the data: mammary adenocarcinoma tissue has been shown to express high levels of BC200 RNA in five different patients, while, for example, colon adenocarcinomas were never found to be positive in this assay. This points to the ability of BC200 RNA to act as a broad spectrum marker for a variety of tumors and neoplastic diseases.

TABLE 1 EXPRESSION OF BC200 RNA IN DIFFERENT HUMAN TUMOR TISSUES AND DIFFERENT CELL TYPES

TISSUE SOURCE	NO. OF CASES	CELL TYPE	BC200 EXP.
HYPOPHARYNX	1	EPITHELIAL TUMOR	-
TONGUE	1	SQUAMOUS CELL CARCINOMA	+
ESOPHAGUS	1	EPITHELIAL CARCINOMA	+
STOMACH	2	ADENOCARCINOMA	-
	1	TUBULAR ADENOCARCINOMA	+
LIVER	4		-
RECTOSIGMOID COLON	2	ADENOCARCINOMA	-
HEMICOLECTOMY	1	ADENOCARCINOMA	-
COLON	5	ADENOCARCINOMA	-
KIDNEY & ADRENAL GLAND	1	CLEAR CELL	-
BLADDER	1	TRANSITIONAL	-
BREAST	5	ADENOCARCINOMA	+++
LUNG	1	SQUAMOUS	+++
	2	ADENOCARCINOMA	-
PARTOID GLAND	1	MUCOEPIDERMOID	+++
THYROID GLAND	1		-
MUSCLE & SKIN	1	SARCOMA	+
LUNG METASTASIS (MELANOMA)	1	MELANOMA	++
OVARIAL	1	PAPILLARY CARCINOMA	+
CERVIX	1	ENDOTHIAL ADENOCARCINOMA	+
NON-HODGKIN LYMPH.	1	T CELL TYPE	-

SEQUENCE LISTING

1. GENERAL INFORMATION

(i) APPLICANT: Henri Tiedge and Jurgen Brosius

(ii) TITLE OF INVENTION: BC200RNA, Probes Therefor and the Use Thereof

(iii) NUMBER OF SEQUENCES: 13

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(v) COMPUTER READABLE FORM:
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(C) OPERATING SOFTWARE: DOS VER. 5.0
(D) SOFTWARE: WP 5.1

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(A) APPLICATION NUMBER:
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(B) FILING DATE:

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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 200
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: MRNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: human
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE: neocortex
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:
(A) NAME/KEY: human BC200 RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GGCCGGGCGC GGUGGCUCAC GCCUGUAAUC CCAGCUCUCA GGGAGGCUAA GAGGCGGGAG 60

GAUAGCUUGA GCCCAGGAGU UCGAGACCUG CCUGGGCAAU AUAGCGAGAC CCCGUUCUCC 120

AGAAAAAGGA AAAAAAAAAA CAAAAGACAA AAAAAAAAUA AGCGUAACUU CCCUCAAAGC 180

AACAACCCCC CCCCCCCUUU 200

(3) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: MRNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: human
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE: neocortex
(G) CELL TYPE:
(H) CELL LINE:

(I) ORGANELLE:

(ix) FEATURE:

(D) OTHER INFORMATION: unique sequence from human BC200 RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

UAAGCGUAAC UUCCCUCAAA GCAACAACCC CCCCCCCCCU UU 42

(4) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(vi) ORIGINAL SOURCE:

(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:

(D) OTHER INFORMATION: probe for unique sequence from human BC200 RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

TTTATTCGCA TTGAAGGGAG TTTCGTTGTT 30

(5) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:

(D) OTHER INFORMATION: primer for human BC200 RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GTTGTTGCTT TGAGGGAAG 19

(6) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:

(I) ORGANELLE:

(ix) FEATURE:

(D) OTHER INFORMATION: amplification primer for t-tailed human BC200 cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GCCTTCGAAT TCAGCACCGA GGGAAGTTAC GCTTA 35

(7) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:

(D) OTHER INFORMATION: amplification primer for t-tailed human BC200 cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GCCTTCGAAT TCAGCACCAA AAAAAAAAAA AAAAA 35

(8) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(vi) ORIGINAL SOURCE:

(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:

(D) OTHER INFORMATION: probe for detection of BC200 RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

TATTCGCATT GAAGGGAGTT T 21

(9) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:

(D) OTHER INFORMATION: amplification primer for BC200RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GCCTTCGAAT TCAGCACC 18

(10) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:

(D) OTHER INFORMATION: amplification primer for BC200RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

AAAAAAAAAN NGCCGGGCGC GGT 23

(11) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:

(D) OTHER INFORMATION: amplification primer for BC200RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GCCTTCGAAT TCAGCACCTT TTTTTTTTT TTTTT 35

(12) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:

(D) OTHER INFORMATION: amplification primer for BC200RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GCCTTCGAAT TCAGCACCAA AATAAGCGTA ACTTCCC 37

(13) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(vi) ORIGINAL SOURCE:

(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:

(D) OTHER INFORMATION: probe for BC200RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CCTCTTAGCC TCCCTGAGAG CT 22

(14) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:

(D) OTHER INFORMATION: ampification primer for BC200RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

CCAGCTCTCA GGGAGGCTAA 20

Claims

1. An oligonucleotide probe which is complementary to and selectively binds with at least a part of the unique domain of human BC200 RNA or to corresponding DNA sequences, said unique domain having the sequence:
UAAGCGUAAC UUCCCUCAAA GCAACAACCC CCCCCCCCCU UU 42
[SEQ ID NO 2].

2. An oligonucleotide probe according to claim 1, wherein the probe has the sequence:
TTGTTGCTTT GAGGGAAGTT ACGCTTATTT 30
[SEQ ID NO 3].

3. An oligonucleotide probe according to claim 1, wherein the probe has the sequence:
TTTGAGGGAA GTTACGCTTA T 21
[SEQ ID NO 7].

4. An oligonucleotide probe according to claim 1, further comprising a detectable label.

5. An oligonucleotide probe according to claim 1, wherein the label is a radioactive, chemi-luminescent, fluorescent or chromogenic label.

6. A method of screening human tissue for the presence of BC200 RNA comprising the steps of:
(a) obtaining a sample of human tissue;
(b) combining the sample with a detection reagent that produces a diagnostic reaction product if BC200 RNA is present in the sample; and
(c) determining whether formation of the diagnostic reaction product has occurred.

7. A method according to claim 6, wherein the detection reagent is an oligonucleotide probe which is complementary to and selectively binds with at least a part of the unique domain of human BC200 RNA, said unique domain having the sequence:
UAAGCGUAAC UUCCCUCAAA GCAACAACCC CCCCCCCCCU UU 42
[SEQ ID NO 2].

8. A method according to claim 7, wherein the probe has the sequence:
TTGTTGCTTT GAGGGAAGTT ACGCTTATTT 30
[SEQ ID NO 3].

9. A method according to claim 7, wherein the probe has the sequence:
TTTGAGGGAA GTTACGCTTA T 21
[SEQ ID NO 7].

10. A method according to claim 6, wherein the diagnostic reaction product is immobilized to facilitate the determination of whether BC200 RNA is present in the sample.

11. A method according to claim 7, wherein the probe includes a detectable label.

12. A method according to claim 7, further comprising the steps of converting RNA present in the tissue sample to DNA and selectively amplifying the DNA in the sample to increase the number of DNA sequences corresponding to BC200 RNA, if present, wherein the oligonucleotide probe is complementary to the amplified DNA sequence.

13. An oligonucleotide probe which is complementary to and selectively binds with at least a part of the Alu-repetitive Domain of human BC200 RNA, or to corresponding DNA sequences, wherein the part of the Alu-repetitive domain to which the probe is complementary includes the bases at positions 48 and 49 of the human BC200 RNA sequence.

14. An oligonucleotide probe according to claim 13, wherein the probe has the sequence:
CCTCTTAGCC TCCCTGAGAG CT 22
[SEQ ID NO 12].

15. An oligonucleotide probe according to claim 13, wherein the probe has the sequence:
CCAGCTCTCA GGGAGGCTAA 20
[SEQ ID NO 13].

16. An oligonucleotide probe which is complementary to and selectively binds with at least a portion of the A-rich region of human BC200 RNA, or to corresponding DNA sequences, wherein the part of the A-rich region to which the probe is complementary includes the bases at positions 146-148 of the human BC200 RNA sequence.

17. A method for screening non-neuronal human tissue for neoplastic disease comprising:
(a) obtaining a sample of tissue; and
(b) analyzing the sample of tissue to determine if BC200 RNA is present in the sample, wherein the presence of BC200 RNA is indicative of neoplastic disease.

18. A kit for identification of tissue samples containing BC200 RNA, comprising:

(a) a detection reagent that produces a diagnostic reaction product if BC200 RNA is present in the sample; and

(b) a solid support adapted to immobilize the diagnostic reaction product.

19. A kit according to claim 16, wherein the detection reagent is an oligonucleotide probe which is complementary to and selectively binds with at least a part of the unique domain of human BC200 RNA or to corresponding DNA sequences, said unique domain having the sequence:
UAAGCGUAAC UUCCCUCAAA GCAACAACCC CCCCCCCCCU UU 42
[SEQ ID NO 2].

20. A kit for identification of tissue samples containing BC200 RNA, comprising:

(a) a detection reagent that produces a diagnostic reaction product if BC200 RNA is present in the sample; and

(b) amplification primers effective to permit selective amplification of DNA complementary to BC200 RNA.

21. A kit according to claim 18, wherein the detection reagent is an oligonucleotide probe which is complementary to and selectively binds with at least a part of the unique domain of human BC200 RNA or to corresponding DNA sequences, said unique domain having the sequence:
UAAGCGUAAC UUCCCUCAAA GCAACAACCC CCCCCCCCCU UU 42
[SEQ ID NO 2].

22. A kit for identification of tissue samples containing BC200 RNA, comprising:

(a) a detection reagent that produces a diagnostic reaction product if BC200 RNA is present in the sample; and

(b) a labeled reagent that reacts with the diagnostic reaction product to render it detectable.

23. A kit according to claim 20, wherein the detection reagent is an oligonucleotide probe which is complementary to and selectively binds with at least a part of the unique domain of human BC200 RNA or to corresponding DNA sequences, said unique domain having the sequence:

UAAGCGUAAC UUCCCUCAAA GCAACAACCC CCCCCCCCCU UU 42

[SEQ ID NO 2].

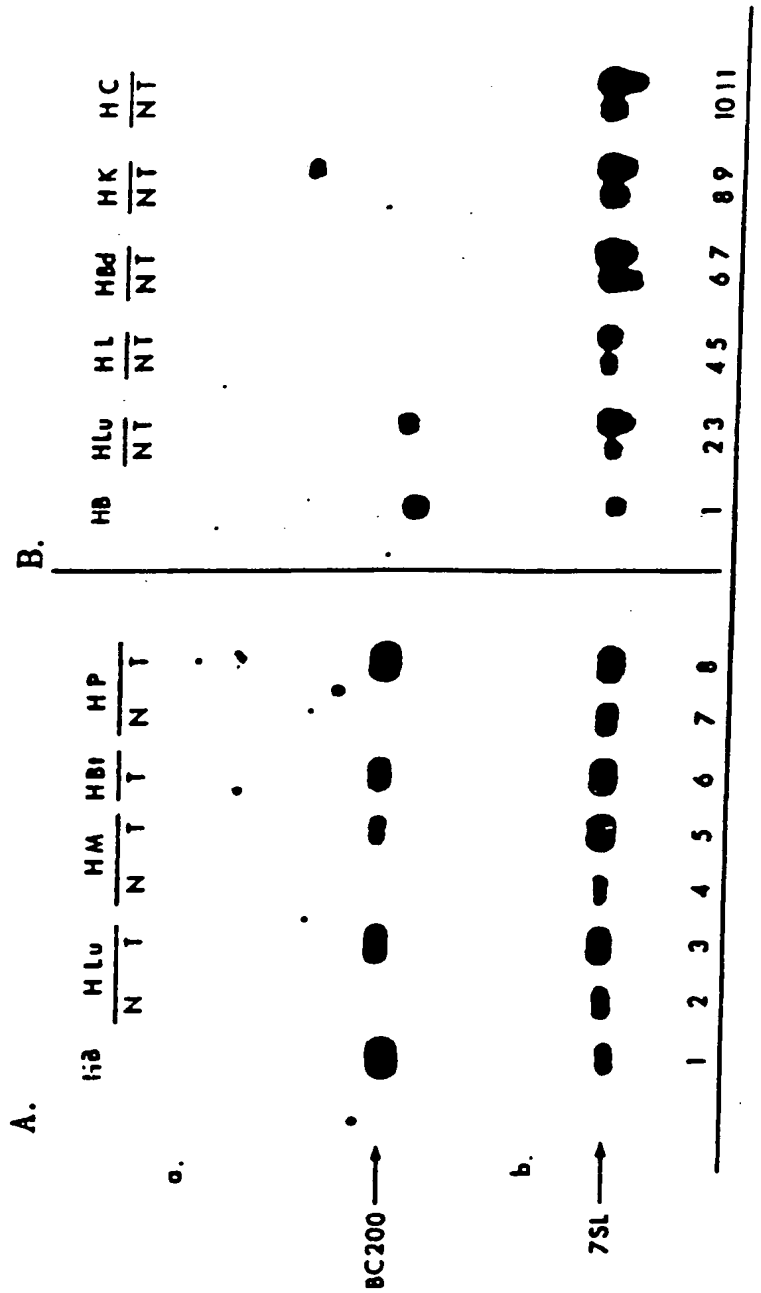

Fig. 1

CORRECTED VERSION*

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(54) Title: BC200 RNA, PROBES THEREFOR AND USE THEREOF

(57) Abstract

The 3'-end of BC200 RNA, commencing at nucleotide 159 has the sequence: UAAGCGUAAC UUCCCUCAAA GCAACAACCC CCCCCCCCCU UU 42, [SEQ ID NO 2]. Oligonucleotide probes in accordance with the invention are complementary to at least a portion of this sequences such that they bind specifically and selectively to human BC200 RNA. The probes are useful in determining the distribution of BC200 RNA in the body and as an indicator of neoplastic diseases in non-neuronal tissue.

* (Referred to in PCT Gazette No. 05/1995, Section II)

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INTERNATIONAL SEARCH REPORT

Internat'l Application No
PCT/US 94/05910

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DISSERTATION ABSTRACT INTERNATIONAL B, vol.53, no.10, April 1993 pages 5080 - 5081 J.A. MARTIGNETTI 'BC1 and BC200 RNA and transcriptional analysis'	1,4,5, 18,20,22
Y	New York City University, NY, US; (1992) 192 pp. Avail.: Microfilms Int., Order No. DA9304701 abstract	6,7, 10-13
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[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
18 November 1994	16. 12. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Hornig, H

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NEUROCHEMICAL RESEARCH, vol.17, no.6, 1992, PLENUM PUBLISHING CORPORATION, NEW YORK, US; pages 591 - 597 W.J. LUKIW ET AL. 'BC200 RNA in normal human neocortex, non-Alzheimer dementia (NAD), and senil dementia of the Alzheimer type (AD)' see page 593, left column, line 1 - page 596, right column, line 13 ---	6,7, 10-13
A	MOL. CELL. BIOL., vol.7, no.9, September 1987, AM. SOC. MICROBIOL., WASHINGTON, D.C. US; pages 3324 - 3327 J.B. WATSON AND J.G. SUTCLIFFE 'Primate brain-specific cytoplasmic transcript of the Alu repeat family' cited in the application the whole document ---	1-23
A	PROC. NATL. ACAD SCI., vol.88, no.6, 15 March 1991, NATL. ACAD SCI., WASHINGTON, DC, US; pages 2093 - 2097 H. TIEDGE ET AL. 'Dendritic location of neural BC1 RNA' cited in the application the whole document ---	1-23
P,X	J. NEUROSCIENCE, vol.13, no.6, June 1993, SOC. NEUROSCIENCE,US; pages 2382 - 2390 H. TIEDGE ET AL. 'Primary structure, neural-specific expression, and dendritic location of human BC200 RNA' see page 2383, left column, line 36 - right column, line 61 see page 2387, right column, line 4 - page 2389, left column, line 51 ---	1-13
P,X	PROC. NATL. ACAD SCI., vol.90, no.24, 15 December 1993, NATL. ACAD SCI., WASHINGTON, DC, US; pages 11563 - 11567 J.A. MARTIGNETTI AND J. BROSIUS 'BC200 RNA: A neural RNA polymerase III product encoded by a monomeric Alu sequence' see page 11564, left column, line 33 - page 11565, right column, line 3 -----	1,4,5

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